Figure 1. Overlap-extension-PCR fragment
with purD deletion

Overlap-extension-PCR fragment
with recA deletion
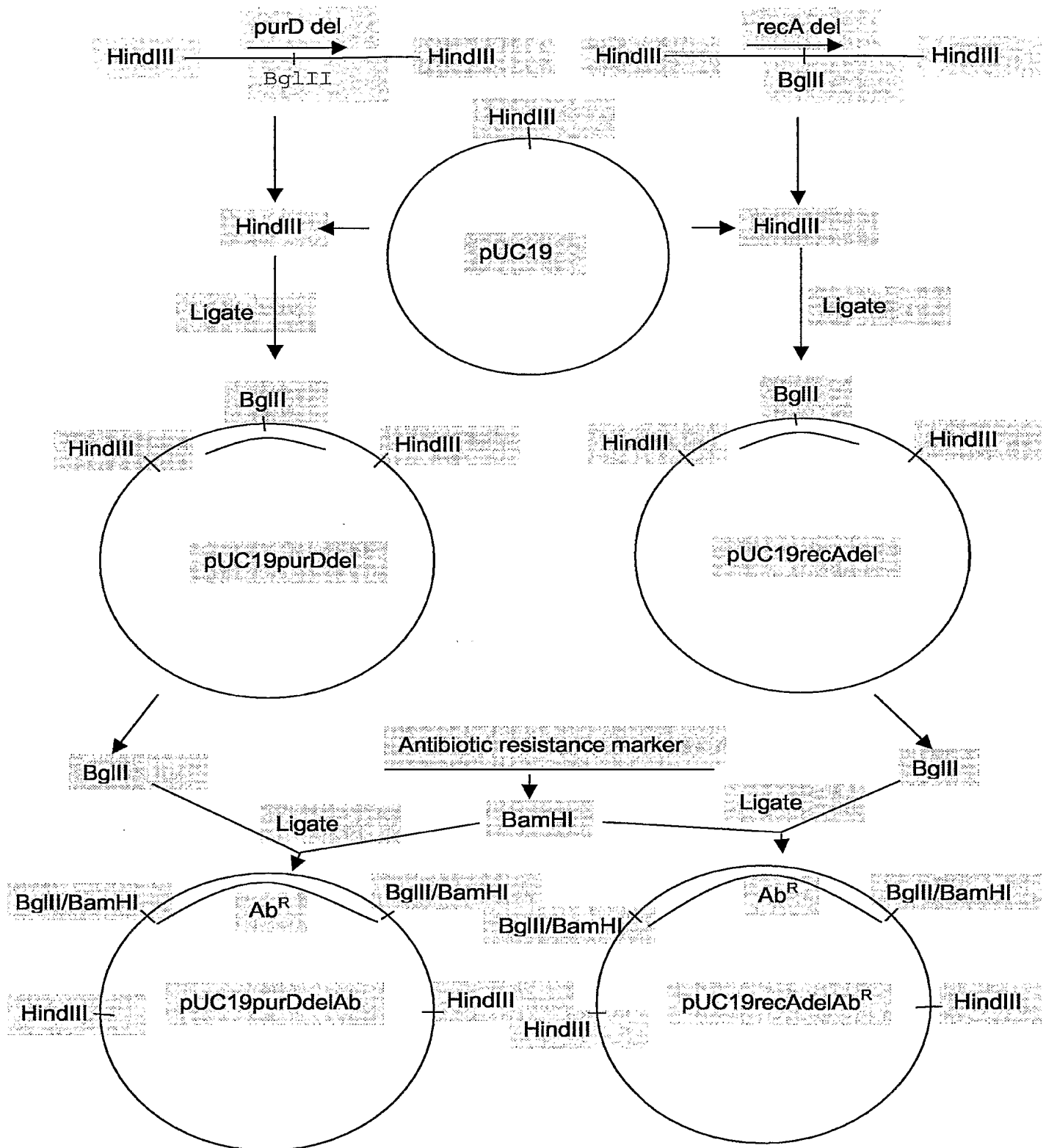
Figure 2A.

```
   1 GTTCGACCAA ACGGCTTGTT GTGCGGTGAA ACATAGCACT CCTTGTGGCG TGGCTTTAGA TGATGATATT TTGCAAGCGT
     >>.......F5........>>           CTTAAGCTTGGA>>.......F13........>>
                                       ------
                                       HindIII

  81 ACCAAAAAGC ACACGACTGC GACCCGATTT CGATTTTTGG TGGCATTGTA ACTTTTAATA AAAAAGTAAC AAAAGCAGTG

 161 GCAGAAAAAT GTAACGAGAT TTTCCTTGAA ATCGTTGCTG CACCGAGCTT TGAGCCAGAG GCTTTGGAAG TTTTTGCTAA

 241 AAAGAAAAAT TTGCGCGTGA TTGAAGTTAA AAATCCATTA AGCGATAAAA TGCAACTCGT GCAAGTAGAT GGCGGATTGC

 321 TCGTGCAAGA AATCGACAAA TCGTTTAGCA ATGATTTTAA AGTAGTAACC GAAAAACAAC CTACCGAAAA GCAACTTTCT

 401 GATTTGGAAT TTGCCATGAA AGTAGTGAAA CATGTAAAGA GCAATGCCAT CGTGGTTGCC ACAAACGGAC AAGCTCTAGG

 481 CGTGGGCACA GGCGAGACTA ATCGTATTTG GGCAGCACAG CAGGCGATTC AGCGTGCAAA GGAAAAAACA CAAGAAAATC

 561 TAGTTTTGGC TTCCGATGCC TTTTTCCCAT TCAGAGATGT GGTAGATTAT GCAGCACAAG AAGGCATTAC AGCCTTGATT

 641 CACCCAGGAG GAAGCATGCG CGACCAAGAG AGCATAGACG CGGCTAATGA ACACGGAATC CCGATGATCA TCAGCGGTAT

 721 GAGACATTTC TTACATTAAA TCAAAAAATC TAAACAATAA TTATCAATAA TTCTAAAACA CAATAAGTAT GAATGCAAAT
                                                                        >>...purD...>

 801 GATTACAAAA AAATACTCAT CGTAGGAAAC GGCGCAAGAG AACACGCCAT CGGGTGGAAA ATTAAACAAG ACCACCCTTC
     >..................................purD.............................................>

 881 TTGCGAGCTT TTCTTTGCGC CAGGAAACGC TGGAACCGAA CAAATTGGAA AAAACATCGT AGCTGAATCT AATTATGGCT
     >..................................purD.............................................>
                    <<.........OE-R..........<<AGATCTGGCGCTACGCTAGAAG
                                               ------
                                               BglII

 961 TAATGCTTTT TGCTCAACAA AATGATATAG ACTTAACGAT TGTAGGTCCA GAAGCAGAAT GGTAGAAGG TATTGTAGAC
     >..................................purD.............................................>

1041 TTGTTTGAAT CCAATCAATT AAGAATTTTT GGTCCAGATA AGCGTGCGGC TAAATTGGAA GGCAGCAAGG CTTTTGCCAA
     >..................................purD.............................................>

1121 AGATTTTATG GAGAAATACG GCGTGCGCAC GGCTTTTGCC AAAAGTTTCA CAATTTTGT AGACGCTAGA GATTATGTAA
     >..................................purD.............................................>

1201 AAGAGCTCAC GCAATTCCCT ATCGTGATCA AAGCCAGTGG CTTGGCAGCA GGAAAAGGTG TGATCATCGT GCACNTACAA
     >..................................purD.............................................>

1281 CTTGAAGCCG AAACTACTTT GCGCAAAATC ATGGAAGACA AAACCTTTGG CGAAGCAGGC AACGAGGTCG TAATCGAGGA
     >..................................purD.............................................>

1361 ATACTTAAAA GGTGTGGAAG TTTCTGTGCT TTCTATCTTT AACCATAAAG AAATTAAAAC TTTCTTGCCT GTAAAAGACC
     >..................................purD.............................................>

1441 ACAAGAAAAT CGGAAAAGGC GAAACAGGAC TCAACACGGG CGGAATGGGC GTAGTGGCTC CTAACCCGCA TTTTACCGAT
     >..................................purD.............................................>

1521 GAGCACATGA AGGAGTTTGA GAAAAACATT TTGCTCCCAA CACAAAAAGG GCTCTTGGCA GAAAAAATGC ATTTTGCAGG
     >..................................purD.............................................>

1601 CATTATTTTC TTTGGGCTTA TGATTACCGA GCATGGTATT TATCTATTGG AATACAACAT GCGATTTGGC GACCCAGAAA
     >...................................purD............................................>

1681 CCGAAGCACT TTTGCCTTTG ATGGAGAATG ATTTAGTAGC CCTCATCGAT TCCGCAATAC ACCAGCAAGA CATTGAACTT
     >..................................purD.............................................>

1761 AAATGGAAAA ACGAACATGC TTGCTGTGTA GTAATGGCGA GCGGTGGCTA CCCAGGCACT TACGAAACTG GTTTTGAAAT
     >..................................purD.............................................>
```

```
1841   CCGAGGATTG AACAAAGTTG ATGTTCCCGT ATTTATTGCA GGAGCCAGAG AAGAAAGTGG AAAAATCTAC ACCACAGGCG
       >.................................purD.......................................>

1921   GGCGCGTGCT CAATGTGGTG GGAACTGGCG CTACGCTAGA AGAAGCCAGA AAAGTGGCTT ACGAAAATAT CCATAAAATC
       >..............................purD.......................................>
                       GAGATCTGG>>......OE-F.......>>
                       ------
                       BglII

2001   AATTTTGATT ATGAATATTA TCGCGAAGAC ATCGGGAAGA TATAATCTCG CTGATTTTTA ACCAAAACAT ATTTAAAAAC
       >....................purD.....................>>

2081   GCTTTTGTTA CTTTTATAAA CAAAGGCGTT TTTCTATTTT TGTGCCACTA TAACATGATT TAACCCATGA AAAAAATACT

2161   AAAAATACTC ATTTTTCTAC TGCTCATTCC TTGGGTTTAT GCCCTGATTT TAATCTTTAT AAATCCACCT ATCACCATTA

2241   CACAGCTGAG CAATTTATCT TATGGTTTCT CCAGAACACA GCTCGCTTAT GATGAAATTC CGGCTAGTGC TAAATGGGCT

2321   GTAATTGCAG CAGAAGACCA GAATTTTGCC ATTCATAATG GCTTTGATTT TAAAGAAATT AAAACCGCCT ACGAGAAAAA

2401   CAAAGCGGGC AAGAAATTGC GTGGCGGGAG CACCCTTTCG CAACAAACTG CCAAAAATGT ATTTTTGTGG CAAGGGCGCA

2481   CTTGGATTAG AAAAGGATTG GAAACCTACT GCACCTTTAT CATCGAAACG CTGTGGAGCA AGGAGCGTAT TTTGCAAGTT

2561   TACCTCAACA ATGCCGAAAT GGGCAAAGGC GTTTATGGCA TAGAGGCAGC GGCGCAATAT TATTTTAAGA AAAACGCCTC

2641   ACAGCTCACG CCTACCGAGA CGGCACGCAT CATTGCCTGC CTGCCCAATC CCAAAAAATA CAATNTAAAC CCGCCAAGTG

2721   CCTACATCTC AAAACGCGGA CAATGGATTC TGCGCCAAGT GCGAAACTTG AAAGGCGATA GGGCTCTGAG CGAGATTGTG

2801   AACACGCCCT AACGCCTGCC TCAACTCTTT GCACACAGTT TACCAACTCT CTGCGAAGAG TTCACAAACT CTTCGCACAC

2881   ACTTCCCCAA GTCTTTGCAA AGAGTTGGGA GATACTTAGG CACAAAAAAA AGGAACCTCA TGAATAGAGG TTCCCTCTTC

2961   CTTAAAAGGA ATAAATAATA ATGTTTTTTA AGCTTTAGGC TTGGCTACTT TTTCAAAGCC TGCTGCCTTC ATGCTATCTA
                                               -------
                                               HindIII

3041   GGATACGCTT GCCTGGGCGG TAGTTTACGC CTACCTTTTT GATTAAGCCC GAATGAAAAT CTTTCTCTGT ATCTGCCGCT
                                                                    <<......R8......<

3121   CCACTGCTTA AAGTGGCATA GAGCGAGCCA AGCTTATCTA AACGAACGAT TTTGCCCGCT GCCAAGGCGT CTTGAATTAC
       <R8.<<AAGCTTAAG
          ------              -------
          HindIII             HindIII

3201   ATTCTCTAGC GCAATGATAA CGCCACGAAT ATCTGCCTCG CTGAGTGCCG AAAACTTCTC GATTTGCTTA ACGAGCTGGT

3281   CTATATCCAT TTCTCCATCG CTTGCCACCA CGGCATAGTA TTTTTGTGGC TCCCCTGGCT TGCTTGGGTT CTACGCTGA

3361   ATTACATTGT ATTTTATGCT CATAATTACT CTATTTTTAA TAGCCTCCCG ATGGATATAA AGTTACGCTA CAATTAGGGT

3441   CTCCATAAGC AAATCTATAC CCCTCTCTTT CATATTCCCT TCTCATTCTT CTTGCTCCAT CTCTCAAGGC ATCCGCTCTA

3521   TTACTGCTAT ACCCCTCCTG AAGAAATGTG TCTGCACTTG AAGAAGAATA TGAAGAGCTA TGAGAATCGT GCAACATAGT

3601   CCAAGCTCCA TCTTGAGCTA TAACATTTGC ATGACATGTA ACACCTATAG TATAATAAAA TCTCCTAGGA GGTTGTGTTC

3681   CACCACCACC TCCAGAGCTA CTACTTTTTT TACATTGTCC ATTTTGGTTA GCATGATTTT GTCCGCCATC ACTTACTAAC

3761   TTCTTAGCTT CTGCTAAGGC TTTTTCTCTT GCTTCTTTT CAGCATCTGC TTGGCTAATT CCACTCACTG CTGTAGCTGT

3841   CGCTTCTTTT TTATAGTTTA CCGAGGTTCC ATAATAGCCA CTACTACAAT TGTTTCTTGT AAAGTTTTTA TTAAAAGATT

3921   GAGTTTGTGT TGAGGTGTAC CCTCCGAAAC CTTTTACTTC TACAGTAAAG GTAGAACTCC CCATGCTTAC GGGGAAGGTG

4001   GCGATAGTAT ACGATTGCCC TGCCGGCATT TGTTTTACTT GATACACTCC ATCTCCTCCC ACTTCTATGC TTGCCGTTAA
```

```
4081  ATTACCACTA CCGCTAAAAG AGCCTTCTGC TATTTTTAGT GTTAAATCAT TTATATCCCC TCCTTGTCCT TTTGCAGAAG

4161  CTTTTGTTAC ACTTACAGCA TCATAAGCTC CTTTTCCATT GGTATAAGGT ATTTATATGG CCAAAC
```

Figure 2B.

```
   1   TAAAGCTGTA AWTCGCTATA AACGCCCTTT AGGATAAAAT CTGCCATTTT TTGCAGTATT TTWATAGCTA AAATTTAGAA
                                 >>.......FrecAOR1.......>>

  81   AACACCATCT CGAGTAAAGG AGCGTGTAGT GCTCGCCATC GTTGAGCGAT TGCCCACCCT CAATTGATTT GGGCGAATAC
              CTTAAGCTT>>.........F6.........>>
              ------
                HindIII

 161   TTGAAATAAA TGGCATCTTC TAGCGACACA TTTTGCGCAG AAATCATGCA AAAAGCCCCG CATAAAAAGC TGAATAAAAA

 241   WGCTAWTYTT CTTGTTTAAA AAAACTCATA AATTCCCCCA AATATAGAAA TATTCTGTGA AAAGTTGCAA TTTATTAACA
                                                                                         <<...<

 321   CTATGTGCTT GCTTTTAATG AAAAAAGTAG ATTATTTTTC CGAATCCGAA AGTTTATTTA CGCCCCATCC GATGCCTAGT
        <..FrecA-4...<<

 401   CCCMSCGATA GCCATGATTA ATACAAATAC AATTAAATCA WATTTTTCMC MTWWACCATA GCACAACACT TGCTAGCTCA

 481   ACGAGTACTA GAGTGGTAAA AAGGATTTTT TGACGATTAT TCATGATTTT ATTTTTCTCA AAGGTAAATA TTTTAAACCA

 561   TAATTTCACA AATCTTAAAA TCTATTTAAA TAATAGAGAA ACCAGAAAAA AATCGTATTT TTACGGAATG AATAAAATGT

 641   TACAAGTAGG CGATAAAATG CCCGATTTCA AAGGTGTAGA CCAATTTGGG AAGGAGCATT CATCTGCCGA TTTCAAAAAT

 721   CAGAAATTAG TCGTTTTTTT CTACCCAAAA GCCAGTACGC CAGGTTGCAC GGCAGAGGCT TGCAACATCA ACGATAATCT

 801   TGATGCGCTA AAAGCACAAG GCTACCAAGT GATAGGCGTG AGTGCAGATT CGGTAGAAAA ACAACGAAAA TTCAGTGATA

 881   AATACGATTT TAAATTCCCT GTGATTGCCG ATGTGGATAA GAAAATTATT GAAGCATTTG GCGTGTGGGG CGAAAAGAAA

 961   TTCATGGGTA AAACCTATGA CGGAATTCAT CGTACGACAT TCATTATTGA TGAAAACGGA GTGGTGGAGC GCGTGATAGA
                                 >>.......F7.......>>
                                         ------
                                          EcoRI

1041   AAAAGTGAAA ACAAAGATC ATACCAATCA AATTTTAAAT TCAGAAAAAT AAAAATATGA GCGAAATAGA CGAAGCGAAA
                                                                    >>.........recA.........>

1121   AGGAAAGCAC TCCAGCTAGT GCTTGATAAA ATGGACAAAA GCTATGGTAA AGGTGCCGTG ATGATGATGG GCGACAAAGC
        >.............................................recA................>
                                                             <<.........OER1.........<

1201   CATAGACGAA AATATTCCAG TAATCCCTAC GGGGTCTCTA GGTTTAGATT TAGCCTTGGG CGTGGGAGGG TATCCGCGCG
        >.............................................recA................................>
        <CGAGATCTCGTGCGTGCGGT
        ------
          BglII

1281   GTAGAATCGT GGAGATTTAC GGTCCAGAAT CTTCTGGTAA AACCACTTTG GCAATTCATG CCATTGCCGA AGCTCAAAAG
        >.............................................recA................................>

1361   TCTGGCGGAA TTGCAGCTTT CATCGATGCA GAGCACGCAT TTGATAGATA TTACGCAGAA AAATTAGGCG TAGATGTTGA
        >.............................................recA................................>

1441   GCATTAATT ATCTCTCAGC CAGATAATGG GGAGCAAGCT TTAGAAATTG CCGATAACTT AATCCGTTCA GGTGCAATTG
        >.............................................recA................................>
                                                 -------
                                                  HindIII

1521   ATATTATTGT AATCGATTCG GTAGCGGCTT TAACGCCAAA GTCGGAAATC GACGGAGATA TGGGCGATTC CAAAATGGGA
        >.............................................recA................................>

1601   TTGCAAGCGC GTTTGATGTC TCAAGCCTTG AGAAAGCTCA CGGGAACTAT CAATAAAACC AAATGTACTG CTATTTTCAT
        >.............................................recA................................>
```

```
1681  CAACCAATTG AGAGAGAAAA TCGGTGTGAT GTTCGGTAGT CCAGAAACCA CAACGGGTGG TAATGCACTT AAATTCTATG
      >............................................recA............................................>

1761  CATCGGTGCG TCTAGACATT CGTCGTTCTA CTCAGATTAA AGATGGGAAC GATGTCATCG AAACTTGAC TCGCGTAAAA
      >............................................recA............................................>

1841  GTAGTGAAAA ACAAAGTAGC TCCGCCATTC CGTAGTGCAG AATTCGACAT TATGTATGGC GAAGGAATCT CTAAAGCAGG
      >............................................recA............................................>
                                                      --------
                                                       EcoRI

1921  CGAGATTTTA GACATTGCTA CCGATTTAGA AATCGTGAAA AAAAGTGGCT CTTGGTATTC TTATGCAGAT ACTAAACTAG
      >............................................recA............................................>

2001  GACAAGGGCG AGATGCCGTG CGTGCGGTAT TGAAAGATAA TCCAGAATTA GCCGAAGAAT TAGAAGAGAA AATTAAAGAA
      >............................................recA............................................>
             CGAGATCT>>.......OEF1.......>>
              ------
              BglII

2081  GAATTAGAGA AAAAATAGAT TTTTTAGTTT TTTTAATTAA ACGAAAAATC CGTTCACTTT GTTGAACGGA TTTTTTTATG
      >.......recA......>>

2161  CTTGAATGAA TTTATTTCCA ATGGATTGAA TAGCCATGCA CTTTTAAATC TTCGCTATCA TAAGTGATTT CTTTGTCGGT

2241  GTTGGGATAG CAAACTTTAA GTCCTGCGTA TTTGGCAATG GCATGTCCTG CGGCAATGTC CCAAAAGTTT ACAGGTCTAA

2321  AGCGGGTGTA CTCCGTAGCC CACCGATCGG CAATTAGCCC AAGTTTGATA ACGCTTCCCA TAGGCTTTGT GCGGAAAATT

2401  TCATGTTCGG ATTTAATTTT TTTGATGTAT TCCTCGGTGC CAGGATCCAT GTGGAATTTG CTACAAAGAA AAGTGTAATC

2481  TTCGGGCAAA TCCATGGTAG GAATTGGCTT GCTGTGTTTC ATCAATTGTT CAAAAAAATC CGATTTCAGA GCCATTTTGT

2561  GCAATTGTTG TTGAGTCCCG ATGAATTTAC GAGAAGGGCA TTTATCGCTA CCGAAATAGA ACAATCCAAG CGATGGGGCG

2641  TACAAAACTC CTAGCTTAGC CGTATTATTC TCAACTAAGC CTAGACACAC GCAATATTCA TCTGTTTTGT TGACAAAATC

2721  CATGGTGCCA TCAATAGGGT CTGCAATCCA ATAGGTGGGC GTATTTCTAA TTTCTTGTAA AGAATCCTTA TCTCCTTCCT

2801  CACTAAAGTA TGGAATGTCT GTAAAGGAAA CATGTTTTTG CAAGATTTTG TTGGCGGCTA AATCTGCACT TGTAACAGGC

2881  GATCCGTCGG CTTTGGTCTC GGTGGAGAAT CCGTTTTGGA TTGTTTTAAA ACCTCTTCGC CAGCAAGTGC TACAGCCCGT

2961  GTTGCGATTT CTAATAAATT CATAATCATT CTTTTATTCT CGAACAAAGT CAAATAATTC TCTGTATTAA AAAATAATTT

3041  TGGCGATAAA AATTAAAATT TATATATAAA ATATCTCTGC AAAAAACCAA ATCAAATATT TAGTGAAATA AAAAAAATTA

3121  GATTGTAAAT TTGCCTTATG TTTTTAGAGA ATACCATAAA TCATAGAAAA AATACGGGCT GGATCGAAGT AATCTGTGGC

3201  TCTATGTTTT CGGGCAAAAC CGAAGAGTTG ATTCGTAGAG TGAAACGAGC CGAATTGGCT GGGCAAAAGG TAGAAATCTT
                                       <<..........R5...........<<AAGCTTAAG
                                                                  ------
                                                                  HindIII

3281  TAAACCCGCA ATTGATAAAC GCTACGATGA GCAAGATGTG GTATCGCATG ATGAAAACAA AAAACAAGCA ACCCCGATTG

3361  AGGCGAGTTC TAACTTGCCC ATTTTAGCAA GCGATTGTGA TGTGGTGGGG ATAGATGAGG CTCAATTCTT TGACGAAGGA

3441  ATTGTTGAGG TGGCAAATCT TTTAGCTAAT TCGGGGAAAA GAATAATTAT TGCGGGATTA GACATGGATT TTAAAGGTCG
                                                                   <<.......RrecAOR1.......<<

3521  TCCATTTGGT CCTATGCCAA ATTTAATGGC GGTAGCGGAA TATGTGACCA AAGTGCATGC AATCTGTGTG AAAACAGGGA
```

table 5

group	no. of chickens	Treatment				Results	
		vaccination at day 1		challenge at day 25	challenge at day 31	% of max airsac score at day 10 (safety)	% of max airsac score at day 38 (efficacy)
1	25	NDV	RecA aerosol	NDV	WT-OR aerosol	2.5	25[b]
2	25	NDV	PurD aerosol	NDV	WT-OR aerosol	7.5	23[b]
3	25	NDV	WT-OR aerosol	NDV	WT-OR aerosol	68	10[b]
4	25	NDV		NDV	WT-OR aerosol	0	47
5	25	NDV		NDV		0	2

[b] Significantly different (p<0.05) compared to the controls (group 11) using two-sided Mann-Whitney U test

table 6

| group | no. of chickens | Treatment | | | | Results |
| | | vaccination | | day 30 | challenge day 35 | % reduction |
			at day 1			
1	15		PurD aerosol	NDV	WT-OR aerosol	no reduction
2	15	NDV	PurD aerosol	NDV	WT-OR aerosol	54%[b]
3	15	NDV		NDV	WT-OR aerosol	no reduction
4	15	MA5		NDV	WT-OR aerosol	no reduction
5	15	MA5	PurD aerosol	NDV	VT-OR aerosol	50%[b]

[b] Significantly different (p<0.05) compared to the controls (group 11) using two-sided Mann-Whitney U test